# PHARMACODYNAMICS AND DRUG ACTION

# Analgesic effects of peripherally administered opioids in clinical models of acute and chronic inflammation

**A series of double-blind, placebo-controlled clinical trials demonstrated that low doses of morphine (0.4, 1.2, and 3.6 mg) administered into the intraligamentary space of a chronically inflamed hyperalgesic tooth produced a dose-related naloxone-reversible analgesia. This analgesic effect is mediated by a local mechanism in the inflamed tissue, because subcutaneous administration of a 1.2 mg dose of morphine failed to elicit an analgesic response. In contrast, submucosal administration of 1.2 mg morphine or 50 μg fentanyl to the site of extraction of an impacted third molar after the onset of acute pain failed to elicit an analgesic response despite demonstration of a sensitive bioassay. These data indicate that peripheral opioid analgesia can be evoked in a model of chronic, but not acute, inflammatory pain, suggesting a temporal dependent mechanism needed for the expression of peripheral opiate analgesia during inflammation in humans. (Clin Pharmacol Ther 2001;70:66-73.)**

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Although the analgesic efficacy of opioids has been established in numerous animal and clinical studies, their precise mechanism and sites of action have not been determined. It is well recognized that opioids produce analgesia through activation of receptors located in the central nervous system (CNS) at both spinal and supraspinal levels.[1,2] However, recent studies suggest that opioids may also activate targets in inflamed tissue, resulting in a peripherally mediated suppression of sensitized nociceptors.

Behavioral studies conducted in rodents indicate that opioids act peripherally to block the hyperalgesia that accompanies prostaglandin $E_2$ injection[3-5] and bradykinin injection[6] and the abdominal writhing[7,8] and tissue inflammation caused by injection of carrageenan[4,9-11] or complete Freund's adjuvant.[12-14] Collectively, these results indicate the following: (1) that peripheral opioid analgesia can be demonstrated when opioids are injected into hyperalgesic tissue, (2) that peripheral opioid analgesia is not evident when opioids are injected into "normal" tissue, and (3) that peripheral opioid analgesia fulfills pharmacologic criteria for a receptor-mediated mechanism. These latter criteria include a dose-response relationship, reversal by a receptor antagonist, and stereospecificity. Several recently published reviews have summarized studies fulfilling these criteria.[15-17]

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Comparatively few clinical studies have used these same pharmacologic criteria to assess the presence of peripheral opioid analgesia in humans. This series of double-blind, placebo-controlled clinical trials evaluated (1) dose-related analgesia after intraligamentary injection of morphine sulfate in patients with pain caused by a chronic dental inflammatory lesion, (2) naloxone reversal of opiate analgesia in this condition, and (3) peripheral opiate analgesia in a model of acute inflammation, the removal of impacted third molars. The endodontic pain model is distinguished from the postsurgical clinical pain model by the chronicity of the inflammation (often greater than 6 months) and its etiologic basis of microbial and immunologic components.[18-20] The results of these multiple parallel clinical studies suggest that peripherally administered opioids are effective only in chronic inflammation with characteristics supportive of a peripheral site of action.

## METHODS

This investigation consisted of 4 double-blind, randomized, placebo-controlled clinical trials conducted at two sites in patients experiencing intraoral pain to evaluate the following hypotheses: (1) Does local administration of opioids produce a peripherally mediated analgesia? (2) Is this analgesia caused by elective activation of opioid receptors? (3) Can these peripheral effects be demonstrated in both acute and chronic inflammation? These hypotheses were tested in two patient populations: The first two studies were performed in patients experiencing dental pain as an acute exacerbation of chronic periradicular periodontitis requiring local anesthetic administration before an endodontic procedure, removal of the dental pulp (see Table I). This is a relatively uncommon event requiring a large dental clinic population to accrue a few subjects per month who fit the study criteria. The second series of two studies, also summarized in Table I, was conducted in subjects experiencing acute pain onset 2 to 4 hours after the surgical removal of impacted third molars, a well-characterized model of acute pain and inflammation.[21,22] Studies at both sites were controlled with both a placebo and a positive control, either local anesthetic administration (studies 1 and 2) or systemic administration of an opioid (studies 3 and 4). Assay sensitivity was confirmed for all 4 studies by the demonstration of a significant difference between the placebo group and the positive control.

Study 1 was a dose-response study in which patients were injected with equal volumes of either saline solution placebo, local anesthetic (2% mepivacaine with 1:20,000 levonordefrin), or morphine sulfate (0.4, 1.2, or 3.6 mg) via a 30-gauge needle inserted into the intraligamentary space of the inflamed tooth (ie, the space between the root of the tooth and the surrounding alveolar bone). Study 2 was a double-dummy design evaluating the specificity and site of action of peripheral morphine analgesia. Patients received both an intraligamentary injection and a subcutaneous injection in the forearm to evaluate whether the drug effect observed after local intraligamentary injection could be reproduced by systemic subcutaneous injection. Treatment groups (Table I) were administered either intraligamentary morphine, subcutaneous morphine, intraligamentary morphine and naloxone, intraligamentary local anesthetic, or saline solution placebo. Patients in studies 1 and 2 were selected from patients with pain who came to the University of Minnesota Dental School Clinic. Subjects were informed of the possible risks of endodontic therapy and the investigational treatments, and they signed a consent form approved by the university's institutional review board. The inclusion criteria for the study were (1) elective root canal therapy for pain originating from a nonvital tooth, (2) an American Society of Anesthesiologists class I or II physical status, (3) literacy at the 8th grade level, and (4) provision of signed informed consent form. Patients were excluded if they were included in any of the following categories: (1) younger than 18 or older than 65 years, (2) analgesic ingestion within the last 4 hours, (3) history of allergy to or abuse of opioids, or (4) pregnancy or current breast-feeding.

Patients in studies 1 and 2 completed a 4-point category pain scale (none, mild, moderate, severe) and a 100-mm visual analog scale for pretreatment pain and were randomly administered drugs as described. After the injections, pain scores were recorded at 2, 5, 10, 15, 20, 25, and 30 minutes. After the 30-minute evaluation, all patients received a local anesthetic (1.8 mL of 2% mepivacaine with 1:20,000 levonordefrin [INN, corbadrine]) infiltrated on the buccal and palatal surface of the teeth in the maxilla or by nerve block of the inferior alveolar and long buccal blocks for teeth in the mandible. Appropriate dental treatment was then performed.

Studies 3 and 4 were conducted in the Pain Research Clinic at the National Institutes of Health with patients meeting similar inclusion and exclusion criteria undergoing the surgical removal of a lower partial or full bony impacted third molar. Subjects were informed of the possible risks of oral surgery and the investigational treatments, and they signed a consent form approved by the National Institute of Dental and Cra-

**Table I.** Summary of experimental design and treatment groups for evaluating peripherally administered opioids

| *Study* | *Clinical pain model* | *Treatment group* | | *No.* |
|---|---|---|---|---|
| | | *Peripheral drug* | *Systemic drug* | |
| Morphine dose-response | Chronic periradicular inflammation | 0.4 mg morphine* | — | 7 |
| | | 1.2 mg morphine* | — | 7 |
| | | 3.6 mg morphine* | — | 7 |
| | | 2% mepivacaine* | — | 7 |
| | | Saline placebo* | — | 7 |
| | | | | 35 |
| Morphine site of action and receptor specificity | Chronic periradicular inflammation | 1.2 mg morphine* | Saline placebo subcutaneously | 15 |
| | | Saline placebo* | 1.2 mg morphine subcutaneously | 15 |
| | | 1.2 mg morphine* plus 0.4 mg naloxone* | Saline placebo subcutaneously | 15 |
| | | 2% mepivacaine* | Saline placebo subcutaneously | 15 |
| | | Saline placebo* | Saline placebo subcutaneously | 15 |
| | | | | 75 |
| Analgesic efficacy of peripheral morphine | Acute postsurgical inflammation | 1.2 mg morphine† | Saline placebo intravenously | 12 |
| | | Saline placebo† | 1.2 mg morphine intravenously | 15 |
| | | Saline placebo† | 0.1 mg/kg morphine intravenously | 15 |
| | | Saline placebo† | Saline placebo intravenously | 16 |
| | | | | 58 |
| Analgesic efficacy of peripheral fentanyl | Acute postsurgical inflammation | 50 μg fentanyl† | Saline placebo intravenously | 19 |
| | | Saline placebo† | 50 μg fentanyl intravenously | 32 |
| | | Saline placebo† | Saline placebo intravenously | 20 |
| | | | | 71 |

*Administered by injection of 0.2 mL into the intraligamentary space on the mesial and distal areas of the inflamed tooth.
†Administered at pain onset by infusion via a polyethylene tubing (PE50) placed submucosally during surgery.

niofacial Research Institutional Review Board. Subjects were premedicated with intravenously administered midazolam (mean dose, 2.8 ± 0.5 mg) and had one mandibular third molar removed while they were under local anesthesia consisting of 2% lidocaine with 1:100,000 epinephrine (mean dose, 75.3 ± 10.6 mg). At the completion of surgery, polyethylene tubing (size PE 50) was placed submucosally into the extraction site under the flap previously raised for the surgical extraction. Lactated Ringer's solution (pH 6.2) was administered at the rate of 10 mL/min to keep the line patent until the onset of acute pain, which was characterized as moderate or severe by the patient on the 4-point category scale and a minimum of 35 mm on the 100-mm VAS. Hemostasis was evident over the first 30 minutes after surgery, and no fluids other than the lactated Ringer's infusion and the drug vehicle were introduced into the surgical site until pain onset, usually 90 to 120 minutes after operation. Drug or saline placebo was then infused either through the polyethylene tubing or intravenously over 10 minutes in the morphine study and over 15 minutes in the fentanyl study. Subjects were questioned at 15-minute intervals for up to 180 minutes for the presence of mandibular anesthesia, the intensity of postoperative pain as measured by category scale and VAS, and the presence of any side effects.

In study 3 morphine sulfate was randomly allocated to 4 groups of subjects: 1.2 mg morphine submucosally/placebo intravenously, placebo submucosally/1.2 mg morphine intravenously, placebo submucosally/0.1 mg/kg morphine intravenously, or placebo both submucosally and intravenously. The 1.2 mg dose of morphine was selected on the basis of the replicate demonstrations of efficacy for chronic inflammation and to avoid possible absorption of a higher dose leading to a systemic site of action. In study 4, 50 μg fentanyl was randomly administered either submucosally or intravenously with parallel saline placebo to result in the following 3 groups: 50 μg fentanyl intravenously/placebo submucosally, placebo intravenously/50 μg fentanyl submucosally, or placebo both intravenously and submu-

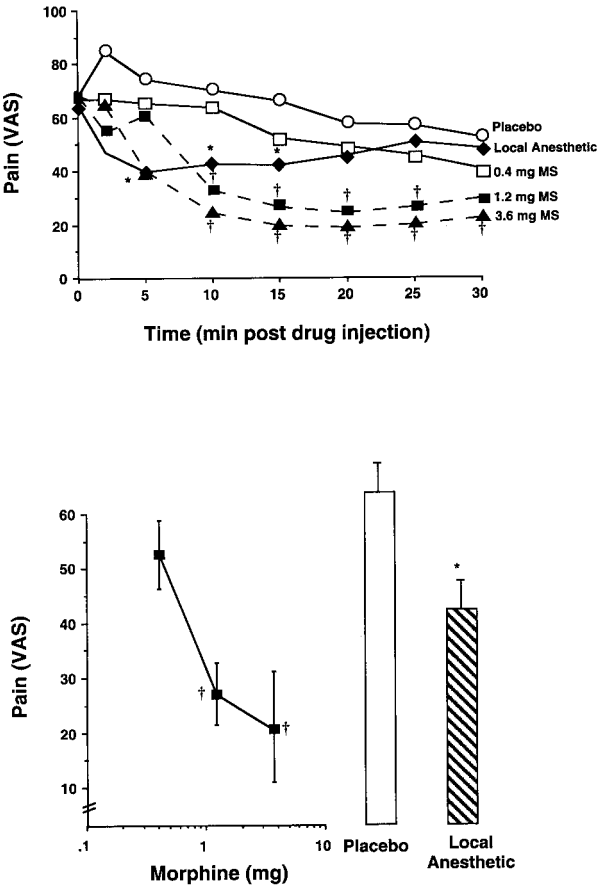



**Fig 1.** Time-response curve for pain as measured on visual analog scale (VAS) in patients with a chronically inflamed tooth after injection into periodontal ligament (PDL) space of placebo, local anesthetic, or morphine (MS) (0.4, 1.2, or 3.6 mg). *Asterisk, P* < .05, versus placebo; *dagger, P* < .01, versus placebo *(upper panel)*. Evaluation of dose-related effect of morphine at 15-minute time point presented in *upper panel* in comparison with placebo and local anesthetic *(lower panel)*.

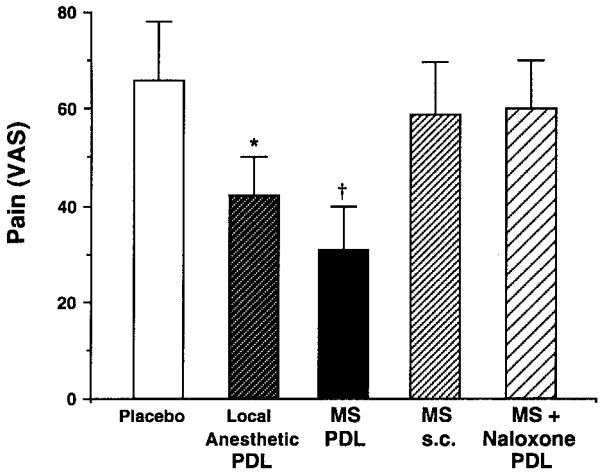


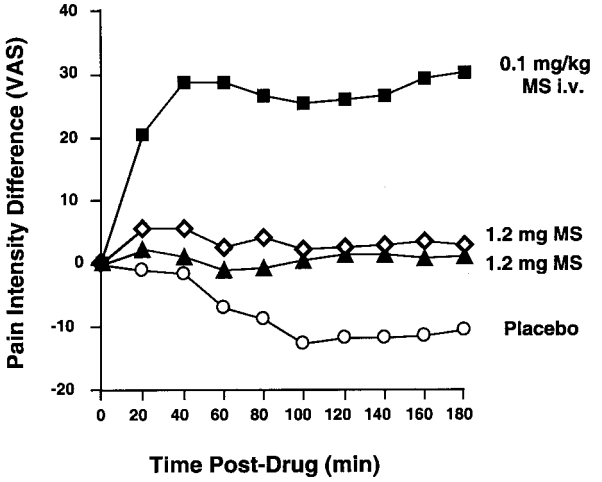

**Fig 2.** Evaluation of site of action (local versus systemic) and naloxone sensitivity of morphine in patients with a chronically inflamed tooth after injection into periodontal ligament (PDL) space of placebo, local anesthetic, 1.2 mg morphine (MS), or 1.2 mg morphine and 0.4 mg naloxone, compared with 1.2 mg morphine administered subcutaneously (s.c). Pain at 15 minutes as measured by 100-mm visual analog scale (VAS). *Asterisk, P* < .05, versus placebo; *dagger, P* < .01, versus placebo.

cosally. The fentanyl dose was selected on the basis of a dose-ranging study (data not shown) with 25, 50, and 100 μg demonstrating that the 50 μg dose was the minimal dose producing analgesia when administered by the intravenous route as a positive control for assay sensitivity.

Parametric data were analyzed by a 2-way repeated measure ANOVA (drug × time) with post hoc analysis by the post hoc Student-Newman-Keuls test. A difference was considered significant if the probability that it occurred by chance alone was <5% (ie, $P < .05$). Data are presented graphically as the mean ± standard error of the mean. The sample size was based on calculations that used a 20% treatment effect, variance at 20 mm on the visual analog scale, and 85% power with a 2-sided test and $\alpha = .05$ level of significance.

## RESULTS

Demographic characteristics and variables related to the endodontic procedure were similar across groups in the first two studies (data not shown). The first study evaluated whether morphine produced a dose-related reduction in endodontic pain after peripheral (ie, periodontal ligament) administration of test drug to a chronically inflamed tooth. Periodontal ligament administration of morphine produced a time-related reduction in endodontic pain as measured on the visual analog scale with peak effects observed over the 15- to 20-minute time periods (Fig 1, upper panel). The dose-response relation at 15 minutes after drug administration (Fig 1, lower panel) demonstrates both linearity with increased dose and separation from the positive control (local anesthetic) and the saline placebo control group at this time point. Similar results were observed for pain data collected with a 4-point category scale (data not shown). The 1.2 mg dose was selected for the subsequent two studies on the basis of the clear separation from placebo and to



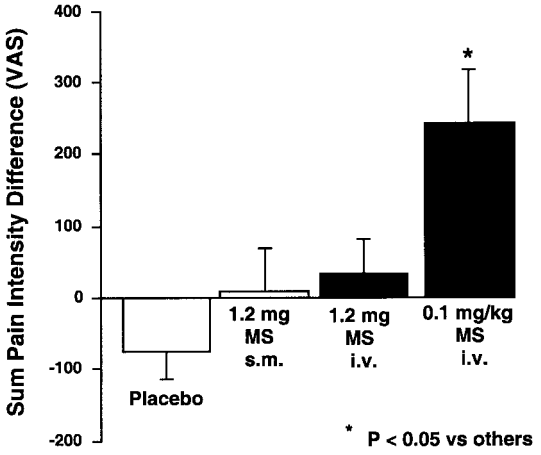
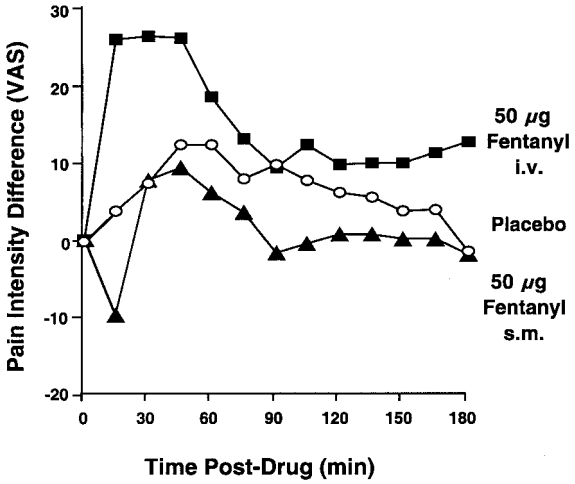




**Fig 3.** Time-response curve for pain relief as measured by difference on visual analog scale (VAS) in pain intensity from predrug status in oral surgery patients after administration of 0.1 mg/kg morphine (MS) intravenously (i.v.), 1.2 mg morphine intravenously, 1.2 mg morphine submucosally (s.m.) at the site of surgery, or placebo given both intravenously and submucosally *(upper panel)*. The pain intensity difference adjusts for variation in starting pain among subjects; a positive value represents analgesia. Total analgesic effect over 180-minute observation period for same 4 groups *(lower panel)*. *Asterisk,* $P < .05$, versus placebo.



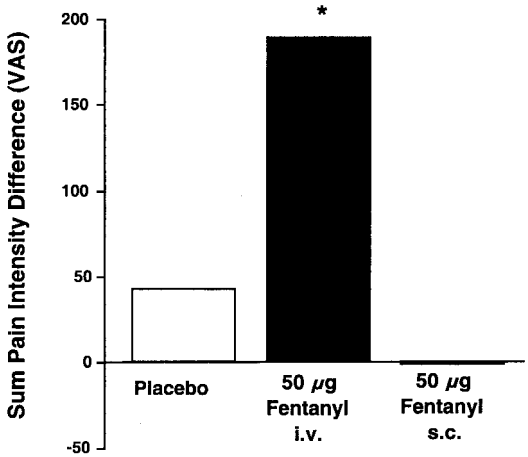




**Fig 4.** Time-response curve for pain relief as measured by difference on visual analog scale (VAS) in pain intensity from predrug status in oral surgery patients after administration of 50 μg fentanyl intravenously (i.v.), 50 μg fentanyl submucosally (s.m.) at the site of surgery, or placebo given both intravenously and submucosally *(upper panel)*. The pain intensity difference adjusts for variation in starting pain among subjects; a positive value represents analgesia. Total analgesic effect over 180-minute observation period for same 3 groups *(lower panel)*. *Asterisk,* $P < .05$, versus placebo.

avoid possible absorption and a systemic effect for the 3.6 mg dose.

To determine the site of opioid receptors mediating the analgesic effect, located either in the peripheral inflamed tissue or at some distant systemic site, the second study compared the analgesic effects of morphine (1.2 mg) given via local periodontal ligament administration or via systemic subcutaneous administration in the volar forearm. The local administration of morphine produced a significant analgesia in comparison with both placebo and the subcutaneous morphine groups (Fig 2). As a further test of pharmacologic specificity,

a separate group of patients were administered a periodontal ligament injection of morphine (1.2 mg) combined with naloxone (0.4 mg). Under these conditions, periodontal ligament administration of morphine produced no significant analgesia compared with administration of placebo. Similar results were observed on a 4-point pain category scale (data not shown).

In the third study the analgesic effect of morphine after the surgical removal of an impacted third molar (Fig 3, upper panel) resulted in a drug effect over time ($F = 5.89$; $P < .01$) with 0.1 mg/kg morphine producing significantly greater analgesia than the other 3 groups ($P < .01$). The sum of the pain intensity difference scores over the 180-minute observation did not demonstrate any difference from placebo for 1.2 mg morphine administered at the extraction site or intravenously (Fig 3, lower panel). Similar results were observed for the category scale (data not shown).

Study 4 used fentanyl to permit better diffusion of opioid through the surgical site as a result of the greater lipid solubility and larger volume of administration (1 mL) in comparison with morphine. Administration of 50 μg fentanyl submucosally after the onset of pain resulted in a nonsignificant increase in reports of pain at the 15-minute observation, presumably because of the opioid-mediated histamine release, but no analgesic effect was detected over the 180-minute observation period (Fig 4, upper panel). Intravenous administration of the same dose resulted in an overall analgesic effect as measured by the sum of the pain intensity difference scores (Fig 4, lower panel), indicating a sensitive bioassay. Similar results were obtained with the category scale (data not shown).

## DISCUSSION

The results of these studies indicate that local administration of low doses of morphine into the ligamental space of an inflamed hyperalgesic tooth produces a significant relief of spontaneous pain. This analgesic effect is locally mediated, since systemic injection of the same dose of morphine fails to elicit an analgesic effect. Further, this peripheral analgesic action of morphine is pharmacologically specific, since it is dose-related and is blocked by concurrent administration of naloxone. Administration of the same dose of morphine or a systemically active dose of fentanyl for acute pain 2 to 4 hours after oral surgery failed to elicit analgesia despite demonstration of a sensitive bioassay. Collectively, these data support the hypothesis that peripheral opioid analgesia can be evoked in a clinical model of endodontic pain characterized by chronic inflammation but not in a surgical model of acute pain and inflammation.

Although numerous animal studies support the hypothesis of peripheral opioid analgesia,[15-17] the results of clinical trials are mixed. In various models of acute postoperative pain, some[23-26] but certainly not all[27-34] studies were able to demonstrate a peripheral analgesic effect of opioids. The reasons for the disparity in results between the animal models and clinical trials are unclear but may be caused by differences in peripheral mechanism of hyperalgesia, species, or testing methods.

In the first 2 studies we selected a clinical model of hyperalgesia that is distinct from acute postoperative pain. Hyperalgesia caused by postoperative inflammation is probably due to incisional pain, as well as to acute release of nonselective inflammatory mediators.[35] In contrast, inflammation as a result of a chronically infected tooth is characterized by an accumulation of chronic inflammatory cells (eg, lymphocytes, macrophages, and the like), neuropeptides, and factors of microbial origin.[18-20,36] Using the endodontic model of hyperalgesia, we have obtained positive evidence for peripheral opioid analgesia in two current independent trials and in a previous study.[37] Thus it is possible that differences in the detection of peripheral opioid analgesia in clinical trials may be due, at least in part, to time-sensitive processes needed for the expression of peripheral opiate analgesia during inflammation.

This hypothesis is supported by a demonstration that 1 mg of morphine administered with a local anesthetic for a heterogeneous group of dental surgical procedures did not differ from placebo for the first 8 hours after surgery but resulted in significantly less pain and analgesic consumption 12 to 24 hours after surgery.[38] Under inflammatory conditions, the axonal transport of opioid receptors from the dorsal root ganglia to the peripheral nerve endings is enhanced, resulting in an increased number of opioid receptors in the inflamed tissue.[39] Other factors associated with mechanisms of peripherally administered opioids, including infiltration of inflammatory cells into the site of injury[16,40] and disruption of the perineural barrier,[41] are also time dependent and may explain a lack of peripheral opioid effect at early time points afater tissue injury. The time course of peripheral opioid analgesia in inflammation induced by complete Freunds's adjuvant, for example, requires 6 hours before fentanyl antagonizes hyperalgesia and up to 12 hours before perineurial leakage is evident.[41] These factors may contribute to the failure to detect peripherally mediated analgesia in the first few hours after oral surgery, onset of analgesia at later time points,[38] and efficacy comparable with that of local anesthesia in chronic inflammatory conditions such as chronic articular and endodontic lesions.

A failure to demonstrate analgesia in the oral surgery model after peripheral opioid administration may be due to loss of drug from the disrupted surgical site or the relative lack of sensitivity of the oral surgery model to opioids. However, administration of 1 mg ketorolac by the same route and method of administration (ie, submucosal infiltration through PE 50 tubing) resulted in significant analgesia in comparison with saline solution placebo,[42] whereas parallel administration of 30 mg ketorolac intramuscularly resulted in faster onset but approximately the same peak relief. Further, a previous study in the oral surgery model also resulted in significantly greater effects for administration into the surgical site of a low dose of ketoprofen in comparison with the same dose administered orally.[43] These results indicate that the oral surgery model is sensitive to the peripheral effects of low doses of analgesic drugs administered at the site of injury. The opioids in this study were administered after the loss of mandibular anesthesia, usually 90 to 120 minutes from the end of the oral surgery procedure. Hemostasis occurs within the first 15 to 30 minutes after surgery, and no fluids were introduced into the surgical site other than the 10-μg/min flow of lactated Ringer's solution to maintain a patent tubing for drug administration. It is unlikely that the lack of peripheral opioid activity in the oral surgery model could be caused by a dilution at the site of administration. The analgesic effects of opioids have been detected in the oral surgery model for a wide variety of agents, doses, and routes of administration,[44] indicating the ability to detect opioid analgesia during acute inflammation on the day of surgery.

Collectively, these studies indicate that local administration of opioids, at the doses and conditions of these 4 studies, produces significant analgesia by a pharmacologically specific mechanism that is active in chronically, but not acutely, inflamed tissue. Future studies may be directed at clarifying the cellular basis for the delayed onset of the peripheral action of opioids and evaluating the clinical utility of this approach for management of inflammatory pain with extended-duration opioid formulations.

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